

## INTERSPECIFIC HYBRIDISATION IN LADYBIRDS (COL.: COCCINELLIDAE)

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### Introduction

THE MOST COMMONLY used definition of a biological species is based upon effective reproduction. Members of a sexually reproducing species do, or potentially could, inter-breed amongst themselves, but not with members of other species (Mayr, 1963). This definition implies not only that matings occur, but that viable and fertile offspring are produced. Matings between individuals of putatively different species have long interested biologists for their potential in helping to unravel the evolutionary mechanisms of speciation, the nature of barriers to mating and so gene-flow between populations, and their possible role in the origin of new species.

Matings between members of different species are a waste of reproductive resources for both partners. The waste for females is generally greater than for males, because females produce large, heavily resourced gametes, while males produce small gametes containing little more than a nucleus and a "tail". However, the cost to males is not negligible. Not only does a male that mates with a female of another species waste gametes, he wastes energy in mating, he wastes time that might be used in more productive ways such as feeding, resting or courting more appropriate females, and, as he is less mobile while copulating, he may be more prone to predation. He also runs the risk of contracting disease from his mating partner. Of course females are liable to these latter costs as well.

Ladybirds that mate with non-conspecific partners suffer all these costs. Mating in *Adalia bipunctata*, a highly promiscuous species, generally lasts for several hours. Both sexes expend considerable energy while mating. The female almost invariably shows rejection behaviour when mounted by a male, stretching up with her back legs, kicking back at the male's genitalia and running while swaying her abdomen from side to side. This rejection behaviour may last for many minutes before the female accepts the male. The male has to cling on while the female is trying to dislodge him. Thereafter, he is energetic throughout the copulation, twisting in a corkscrew motion during the first phase of mating, and rocking rapidly from side to side at regular intervals later (Ransford & Majerus in prep.). Mating ladybirds are less mobile than those that are not mating, and, in *Coccinella 7-punctata* at least, are consequently more prone to parasitisation by the braconid wasp *Dinocampus coccinellae* (Majerus, unpublished data). Several species of coccinellid (*A. bipunctata*, *A. 10-punctata*, *C. 11-punctata*, *Hippodamia convergens*, *Tytthaspis 16-punctata*, and two African species, *Exochomus fulvimanus* and *E. concaius*) are also known to bear a

mite, *Coccipolipus hippodamiae*. This mite is transmitted from one ladybird to another during mating and has a detrimental effect on host fecundity and fertility (McDaniel & Morrill, 1969; Husband, 1984; Majerus, 1994; Hurst *et al.*, 1995; Webberley *et al.*, in prep.).

Given these potential costs to mating with a member of another species, selection should promote mechanisms of species recognition in animals. That this is the case is supported by the existence of a huge array of such mechanisms and by the fact that interspecific matings are very rare in animals. However, they do occur occasionally. This paper contains details of a number of interspecific matings involving ladybirds, and discusses the reasons for the occurrence of this apparently inept behaviour.

### Observations of hybrid matings

The records of interspecific matings contained herein comprise three categories defined by the situation of the mating ladybirds. Some observations are of ladybirds mating in the field. Others are of matings between individuals that had recently been collected and had been placed together in the same container. The third category involves ladybirds that were maintained in the laboratory, under specific conditions, for some period before being placed together specifically to determine whether they would copulate.

#### *Field observations*

Details of 18 field observations of interspecific matings are given in Table 1. Most of the pairs concerned were collected. These were kept in captivity to determine the outcome of the copulation. The ladybirds were kept individually in Petri-dishes, and fed daily on pea aphids *Acyrtosiphon pisum*, black bean aphids *Aphis fabae* or oyster scale *Pseudochermes fraxini* depending on the food preference of the female involved. The dishes containing females were examined for eggs daily. If eggs had been laid, the female was removed to a clean dish. Eggs were counted and monitored for signs of embryonic development. Small pea aphids were added to any dishes containing eggs that showed signs of development. If the eggs hatched, larvae were reared on pea aphids, using techniques previously described (Majerus *et al.*, 1989). The external morphology of fourth instar larvae and pupae was examined to compare with that of the parental species. Any progeny that reached adulthood were examined in detail. The sex, pronotal and elytral colour patterns and other external morphological features were noted while these ladybirds were alive. Attempts were then made to back-cross them to an opposite sex individual of the parental species that they most resembled. In the case of progeny that resembled *C. 7-punctata*, adults were overwintered before back-crossing, as most British individuals of this species require a dormant period before becoming reproductively mature. Eggs from any successful matings were collected and their hatch rates

recorded as an indication of fertility level. Once the ladybirds had died, their genitalia were examined to check sex and look for abnormalities that might be indicative of an interspecific hybrid origin.

Brief details of the outcomes of these treatments for each of the pairs collected are given in Table 1.

**Table 1. Records of hybrid matings observed in the field.**

+ Indicates that copulation was already in progress when the pair was first seen.

\* Suspected that the progeny were the result of the female having previously mated with a male of her own species.

| Location               | Date       | Male                   | Female                 | Mins. mating | No. of eggs laid | Egg fate                       |
|------------------------|------------|------------------------|------------------------|--------------|------------------|--------------------------------|
| Cambridge              | May 1984   | <i>A. bipunctata</i>   | <i>A. 10-punctata</i>  | 43+          | 14               | Infertile                      |
| Abingdon, Oxon         | July 1984  | <i>A. bipunctata</i>   | <i>A. 10-punctata</i>  | 26+          | >100             | <i>A. 10-punctata</i> progeny* |
| Exeter, Devon          | May 1985   | <i>A. bipunctata</i>   | <i>A. 10-punctata</i>  | all day      | ?                | (Brakefield, pers. comm.)      |
| Bedford, Beds.         | July 1986  | <i>A. bipunctata</i>   | <i>A. 10-punctata</i>  | 30+          | ?                | (Hardimann, pers. comm.)       |
| Totnes, Devon          | July 1987  | <i>A. bipunctata</i>   | <i>A. 10-punctata</i>  | 126+         | >100             | <i>A. 10-punctata</i> progeny* |
| Totnes, Devon          | July 1987  | <i>A. bipunctata</i>   | <i>A. 10-punctata</i>  | 83           | 58               | Infertile                      |
| Calais, France         | Sept. 1989 | <i>A. bipunctata</i>   | <i>A. 10-punctata</i>  | 24           | None             |                                |
| Keele, Staffs.         | June 1991  | <i>A. bipunctata</i>   | <i>A. 10-punctata</i>  | 154+         | >200             | <i>A. 10-punctata</i> progeny* |
| Cardiff                | June 1985  | <i>A. 10-punctata</i>  | <i>A. bipunctata</i>   | 8+           | 5                | Infertile                      |
| Cambridge              | June 1990  | <i>A. 10-punctata</i>  | <i>A. bipunctata</i>   | 49+          | >200             | <i>A. bipunctata</i> progeny*  |
| Hyde Park, London      | May 1994   | <i>A. 10-punctata</i>  | <i>A. bipunctata</i>   | 38+          | 196              | 5 hybrid progeny               |
| Calais, France         | Sept. 1989 | <i>C. 7-punctata</i>   | <i>C. 11-punctata</i>  | 64+          | None             |                                |
| Cambridge              | June 1993  | <i>C. 11-punctata</i>  | <i>C. 7-punctata</i>   | 120+         | 287              | <i>C. 7-punctata</i> progeny*  |
| Lincoln                | July 1994  | <i>C. 11-punctata</i>  | <i>C. 7-punctata</i>   | 47           | 284              | <i>C. 7-punctata</i> progeny*  |
| Clervaux, Luxembourg   | July 1995  | <i>C. 5-punctata</i>   | <i>C. 7-punctata</i>   | 58+          | 127              | <i>C. 7-punctata</i> progeny*  |
| Lakenheath, Suffolk    | Apr. 1991  | <i>E. 4-pustulatus</i> | <i>C. 7-punctata</i>   | 17           | None             |                                |
| Chobham Common, Surrey | May 1991   | <i>E. 4-pustulatus</i> | <i>C. bipustulatus</i> | 102          | 14               | Developed but did not hatch    |
| Lakenheath, Suffolk    | May 1995   | <i>C. bipustulatus</i> | <i>E. 4-pustulatus</i> | 36+          | 71               | Developed but did not hatch    |

*Observations of recently-collected ladybirds*

Observations of interspecific matings between recently-collected individuals involve ladybirds that were either put into the same container in the field, or ladybirds that were sent in to the Cambridge Ladybird Survey and were placed in the same container, when they were unpacked, before sorting. In all cases, the matings involved took place less than 72 hours after the ladybirds were collected from the wild. The ladybirds involved were treated in the same way as those collected having been observed mating in the wild. Details of these pairings appear in Table 2.

**Table 2. Records of hybrid matings between recently collected coccinellids**

1. A female *C. 5-punctata* and a male *C. 11-punctata* sent in the same box from the Spey Valley, Scotland, in June 1987 by Patricia Duncan, mated soon after arrival. They subsequently remated on three occasions. The female *C. 5-punctata* produced eggs, however, all the progeny were normal *C. 5-punctata*, the ensuing stock being maintained for two more generations without producing any abnormal descendants.
2. Male *E. 4-pustulatus* repeatedly attempted to mate with both a *C. renipustulatus* (sex not determined) and a male *A. bipunctata*. Mating attempts were apparently unsuccessful (Davies, *pers. comm.*).
3. Male *C. bipustulatus* and female *E. 4-pustulatus* collected Lakenheath, May 1987, mated within a few minutes of collection and stayed *in copula* for over two hours. No eggs resulted.
4. Male *C. 7-punctata* placed in container with a number of *C. 11-punctata* collected earlier the same day, attempted to mate with several of them, and then apparently succeeded in copulating with one, remaining *in copula* for approximately 45 minutes. Some 73 eggs were produced, but all progeny were normal *C. 11-punctata*.
5. A female *A. bipunctata* and a male *A. 10-punctata* collected from overwintering sites in Cambridge, 11 December 1991, mated the following day while still in collecting box. The pair copulated for 75+ minutes. No eggs resulted for 11 days. Thereafter, a few infertile eggs were laid.
6. Male *A. oblitterata* mated with a female *C. 7-punctata* in the laboratory, for over an hour, shortly after being collected from the wild. No fertile eggs resulted.
7. A sample of *T. 16-punctata*, swept from grass with a few *C. 7-punctata*, at King's Forest, Suffolk, in June 1994, were placed in the same box. A male *T. 16-punctata* mated with a female *C. 7-punctata* shortly afterwards. The pair were isolated to allow close scrutiny. The pair remained *in copula* for 71 minutes. the female did not appear to reject the male.
8. A male *C. magnifica* and a female *C. 7-punctata* collected together in Germany by Hinrich Schulenberg, mated. The pair was sent to John Sloggett in Cambridge. No eggs resulted (Sloggett, *pers. comm.*).



*Laboratory observations*

It is known that isolating *A. 10-punctata* or *A. bipunctata* adults from opposite sex conspecific individuals, for a period of two or more weeks, while keeping them active (temperature 12-28°C) and well-fed on an appropriate aphid diet, will lead these individuals to mate with partners of the other species more readily than is normal (Lusis, unpublished data communicated by I.A. Zakharov; Ireland *et al.*, 1986; Majerus & Kearns, 1989; O'Donald & Majerus, 1993; Majerus, 1994). It was considered possible that this procedure might induce other coccinellid species to copulate. Therefore, at various times over the last ten years, when culturing two or more species of coccinellid, the chance to test this possibility has arisen. In these cases, known virgin females of one species that were in reproductive condition and had been fed on an excess of a principal food (*sensu* Hodek, 1973) for at least two weeks, were offered males of a different species in Petri-dishes, a single pair being placed in each dish. The ladybirds were kept under observation and their behaviour recorded. Copulations that occurred were timed. Pairs were separated after parting, and the females were treated as described above.

The details of pairs of species tested and the results obtained are given in Table 3.

**Results and discussion***Matings observed in the wild*

Eighteen interspecific hybrid pairs were recorded in the wild. Of these, 15 were between congeneric species. Of the others, two were between members of the sister genera *Exochomus* and *Chilocorus*. Only one pair involves species that would not be considered closely related, that being the mating between *E. 4-pustulatus* and *C. 7-punctata*.

The initial mounting of the female by the male was only observed in five of the matings. It was notable that in none of these cases did females show strong initial rejection of the male, and insertion of the siphon occurred within two minutes. In the mating between *E. 4-pustulatus* and *C. 7-punctata*, the female began to show vigorous rejection behaviour (kicking back with her hind legs) some 16 minutes after being mounted. The male disengaged after about a minute. The lack of initial rejection of males by females suggests that the females may have been reproductively mature, and ready to oviposit, but that they had been unmated for some period. Females (whether virgin or not) that have been kept isolated from males for a significant period show less rejection behaviour than those that have mated in the last two or three days (Ireland *et al.*, 1986). In the case of the female *C. 7-punctata* that began to reject the *E. 4-pustulatus* male after initially appearing to accept him, her response may have been triggered by some genitalial incompatibility.

**Table 3. Results of attempts to induce interspecific hybrid matings between coccinellids by isolating females for two weeks or more before offering a male of a different species**

| Male                     | Female                   | No. of matings                      | Mean mating duration (mins.) | No. of eggs | Notes on offspring                                         |
|--------------------------|--------------------------|-------------------------------------|------------------------------|-------------|------------------------------------------------------------|
| <i>A. bipunctata</i>     | <i>P. 14-punctata</i>    | 2                                   | 117                          | 54          | No development.                                            |
| <i>P. 14-punctata</i>    | <i>A. bipunctata</i>     | 1                                   | 83                           | 87          | No development.                                            |
| <i>A. bipunctata</i>     | <i>A. oblitterata</i>    | 5                                   | 96                           | 167         | No development.                                            |
| <i>A. oblitterata</i>    | <i>A. bipunctata</i>     | 2                                   | 138                          | 473         | No development.                                            |
| <i>H. 4-punctata</i>     | <i>H. axyridis</i>       | 4                                   | 164                          | 128         | 8 eggs showed signs of development.<br>None hatched.       |
| <i>H. axyridis</i>       | <i>H. 4-punctata</i>     | 2                                   | 214                          | 51          | 3 eggs showed signs of development.<br>None hatched.       |
| <i>A. ocellata</i>       | <i>A. labiculata</i>     | 4                                   | 98                           | 201         | 15 eggs developed.<br>3 hatched. All died in first instar. |
| <i>A. labiculata</i>     | <i>A. ocellata</i>       | 7                                   | 71                           | 154         | 11 eggs developed.<br>6 hatched. All died in first instar. |
| <i>C. 7-punctata</i>     | <i>C. magnifica</i>      | Would not mate in three replicates. |                              |             |                                                            |
| <i>C. magnifica</i>      | <i>C. 7-punctata</i>     | 3                                   | 93                           | 0           |                                                            |
| <i>C. 11-punctata</i>    | <i>C. 7-punctata</i>     | 4                                   | 56                           | 23          | No development.                                            |
| <i>C. 7-punctata</i>     | <i>C. 11-punctata</i>    | 1                                   | 42                           | 0           |                                                            |
| <i>C. renipustulatus</i> | <i>C. bipustulatus</i>   | 1                                   | 45                           | 16          | 2 eggs developed.<br>None hatched.                         |
| <i>C. bipustulatus</i>   | <i>C. renipustulatus</i> | 5                                   | 39                           | 28          | No development.                                            |
| <i>E. 4-pustulatus</i>   | <i>C. bipustulatus</i>   | 3                                   | 71                           | 53          | 14 eggs developed.<br>None hatched.                        |
| <i>C. bipustulatus</i>   | <i>E. 4-pustulatus</i>   | 6                                   | 38                           | 13          | 1 egg developed.<br>It did not hatch.                      |

Thirteen of the pairs produced eggs. The eggs from five of the pairs, three between *A. bipunctata* and *A. 10-punctata* and two between *E. 4-pustulatus* and *C. bipustulatus*, failed to hatch. The eggs from the matings involving *Adalia* species shrivelled and turned orange after about three days, a pattern which usually indicates that eggs are infertile. It is probable that the females involved were virgins, or at least were devoid of sperm from previous matings. The eggs laid, if they were fertilised at all, showed no signs of

development, indicating that zygotes perished very early in embryogenesis. Conversely, some of the eggs from the two matings between *E. 4-pustulatus* and *C. bipustulatus* did begin to develop, turning grey after three days. The deduction in the case of these two pairs is that the eggs produced were the product of the observed hybrid matings, but that the genetic constitution of the species is sufficiently divergent that the zygotes produced suffer developmental breakdown. The possibility that the zygotes failed to hatch because they became cramped in the eggs before they were ready to hatch, as is known to occur in some hybrids between different species of hawkmoth (Newman, 1965) was considered. However, this does not seem probable given that the two pairs were reciprocal, unless the hybrid zygotes were larger than those of either parent.

The remaining eight pairs produced eggs that did hatch. The egg hatch rates of seven of these were high (>70%). Examination of the progeny from these seven crosses revealed all to be apparently normal individuals of the maternal species. The back-crosses all produced high egg hatch rates, and progeny that were normal. The deduction that the females involved in the hybrid matings had previously been mated by one or more conspecific males is obvious. The final cross, between a male *A. 10-punctata* and a female *A. bipunctata* produced 183 eggs of which only five hatched. These were all raised to adulthood. From examination of the larvae and adults it was obvious that these were true hybrids. The fourth instar larvae were paler than those of *A. bipunctata*, but had the abdominal spotting pattern of this species rather than that of *A. 10-punctata*. The patterns of the adults were variable. Two had pronotal patterns similar to those of the typical form of *A. bipunctata*, the others having pronota more like those of *A. 10-punctata*, but with the spots fused together. The ground colour of the elytra (at three weeks old), was orange in all five cases, and was thus akin to that of *A. 10-punctata*. The elytra of one was marked with a single bold black central spot as in typical *A. bipunctata*. The other four had small dark-brown dots on the elytra. Three had six of these dots, the other having ten, positioned as in a typical *A. 10-punctata*. The legs of all five hybrids were brown as in *A. 10-punctata*. Externally, two of the progeny appeared male, the other three appearing female.

Attempts to back-cross these progeny failed to produce issue. Three of the individuals (one male and two females) did appear to mate normally, however, none of the eggs laid as a result showed any sign of embryonic development.

Dissection of the two males showed each to have severely under-developed testes. The siphon of both males appeared indistinguishable from that of a normal *A. bipunctata*. The ovaries of the females were variable. In one the ovary was extremely under-developed. In the second the left ovary appeared normal, while the right ovary was only partly developed, and the ovarioles were somewhat distorted. The ovaries of the third appeared to be

normal. The genitalia of the three females were all similar. As with female hybrids of these two species described by Ireland *et al.* (1986), the genitalia were more similar to those of *A. bipunctata* than those of *A. 10-punctata*, but in all three the infundibulum was unique, and readily distinguishable from that of either parental species. It is questionable whether, in back-cross matings, males of either parental species would be able to insert their siphos and manufacture a spermatophore efficiently in one of these female hybrids.

### *Recently collected ladybirds*

The observations of interspecific matings between ladybirds recently collected extend the range of species which will attempt or actually mate together. Again most of the pairings observed were of congeneric species, however, two were between individuals from different genera. Both of these matings involved female *C. 7-punctata*, one pairing with a male *Aphidecta oblitterata*, and the other mating, most incongruously with a male *T. 16-punctata*. Because of the difference in size between the sexes in these two pairings, and in particular the minute size of the male *T. 16-punctata*, the genital contact was examined both during the copulation and at the moment of withdrawal. In both cases the impression was that the male's siphos was fully embedded, an impression that was reinforced as the siphos were withdrawn.

There are two possible explanations for the occurrence of interspecific hybrid matings between recently collected individuals. First, males may be stimulated by the close proximity of, or contact with, other coccinellids, particularly if they have not encountered females in the wild for some time. Reproductively mature male ladybirds are known to mount other coccinellids, and some other beetles, rather indiscriminately of species or sex, or even of whether the individual being mounted is alive or dead (Majerus, 1994). Second, recently collected females placed with males may be prepared to accept non-conspecific partners if the female has not copulated for some time. This may be the case when population density is low, and appropriate food is common so that members of a species do not become concentrated together on a few patches of resource-rich habitat. These two possibilities are not mutually exclusive.

In three of the cases (numbers 1, 3 and 6) it seems probable that what might be termed "frustrated female syndrome" was the primary cause of the interspecific pairings. With respect to pair 1, both *Coccinella 5-punctata* and *C. 11-punctata* were recorded as being rare in the Spey Valley in 1987 (Duncan, *pers. comm.*). Indeed, the female *C. 5-punctata* in question is one of only two recorded from that region since the early 1950s (Majerus & Fowles, 1989; Majerus, 1994). That said, the fact that this female produced normal *C. 5-punctata* progeny suggests that she was not a virgin. In the case of pair 3, the female species, *Exochomus 4-pustulatus*, was unusually scarce in the Lakenheath area in 1987. The fact that the female that accepted a



*C. bipustulatus* male failed to produce any eggs suggests that she was devoid of conspecific sperm. The same argument applies to the case of the female *C. 7-punctata* that mated with *A. oblitterata* (pair 6).

The instances of a male *E. 4-pustulatus* which attempted to mate with both a *Chilocorus renipustulatus*, and a male *A. bipunctata* (pair 2) and of the male *C. 7-punctata* that attempted to mate with several *C. 11-punctata* (pair 4) are both probably the result of male "randiness". Certainly the male *E. 4-pustulatus* attempted to mount any ladybird he came into contact with.

The other two pairings fit less well with either the female frustration syndrome or male randiness explanations. The pairing of an *A. bipunctata* with an *A. 10-punctata* only 24 hours after they were removed from overwintering sites is certainly difficult to explain. Usually female *A. bipunctata* have to be fed for several days after removal from overwintering sites before they mate. This is probably because ovaries that become dormant in the winter have to be reactivated and resourced. The fact that no eggs were laid by the female for 11 days after the mating suggests that the female was not bearing ovaries with mature eggs. The *T. 16-punctata* that mated with a female *C. 7-punctata* was one of 39 collected with five *C. 7-punctata* by sweeping long grass. All 44 ladybirds were placed in the same small perspex container. Both species were very common at the site making the female frustration extremely unlikely. It is possible that the male may have been stimulated by the proximity of conspecific females in the box, and simply mounted the wrong partner. In such a case, the female would usually reject a ladybird of another species. That this female *C. 7-punctata* did not reject the male may be a consequence of his very much smaller size. The female may simply not have realised what was going on.

None of these pairings produced true hybrid offspring. In all cases, either no eggs were laid, or the eggs were infertile, or the eggs were fertile but the progeny produced were normal individuals of the maternal species.

### *Laboratory pairings*

Most of the pairings set up were of congeneric species. The exceptions were crosses of *A. bipunctata* with both *P. 14-punctata* and *A. oblitterata*, and of *E. 4-pustulatus* and the two British *Chilocorus* species. The protocol of keeping females isolated from males for two weeks or longer proved successful in inducing hybrid mating in all cases except one. The general conclusion is that the rejection behaviour that female ladybirds of most species show towards non-conspecific males can be broken down easily. Interestingly, there was some indication that sympatric species mated less readily than those that do not have overlapping distributions. Thus, the pairs of species that hybridised most easily were *Anatis ocellata* and *A. labiculata* from England and the USA respectively, and *Harmonia 4-punctata* and *H. axyridis* from England and Japan respectively. The reason for this may be that part of the female's mate recognition system involves a rejection

response to males of other species that they encounter reasonably frequently in evolutionary time. This part of the recognition system is only likely to extend to sympatric species that share similar habitat requirements.

The one pairing that did not produce a copulation involved female *C. magnifica* that were placed with male *C. 7-punctata*. This failure is of particular interest because the reciprocal pairing did produce matings, although no eggs resulted. *Coccinella magnifica* is a myrmecophile, usually living in close proximity to nests of the wood ant *Formica rufa* (Donisthorpe, 1920; Pontin, 1960; Majerus, 1989). It is morphologically very similar to *C. 7-punctata*, and often occurs with it. Speculation on the evolutionary divergence of these two species has concentrated on the ecological questions of why *C. magnifica* lives close to ants, and why it does not live elsewhere (see Majerus, 1994 for review). Less consideration has been given to the way in which gene flow between the two species was arrested. The fact that female *C. magnifica* would not accept male *C. 7-punctata*, even when the females had been prevented from mating for a considerable period, suggests that the mate recognition system of females of this species is stronger than that of most.

Only two of the successful pairings (*C. magnifica* male x *C. 7-punctata* female and *C. 7-punctata* male x *C. 11-punctata*) failed to produce eggs. However, the eggs from six of the pairings did not show any sign of development, indicating either that the eggs had not been fertilised, or that the zygotes perished early in embryogenesis.

A small proportion of eggs developed from seven of the hybridisations. However, in only two of these, the reciprocal crosses of *A. ocellata* and *A. labiculata* did any of the eggs hatch, and in both instances all the larvae died in the first instar. Interestingly the two reciprocal sets of crosses between non-sympatric congeneric species (*A. ocellata* x *A. labiculata* and *H. 4-punctata* x *H. axyridis*) all showed some embryonic development, perhaps suggesting that the divergence between these species may not be as great as that between *C. 7-punctata* and either *C. magnifica* or *C. 11-punctata*.

#### *When are interspecific hybrid matings likely to occur in the wild?*

The ease with which females can be induced to mate with males from other species suggests that interspecific hybrid matings may occur in the wild whenever females of a species do not encounter males very frequently. This may occur in three situations. First, when a species is at very low density, males and females may simply not come across each other very often. This may be the result of a species either being very rare, or being less rare but distributed widely and thinly across the environment.

Second, there is a period towards the end of the main reproductive season in Britain, but before the new generation of adults have emerged from their pupae and become reproductively mature, when the sex ratio of adults in mating condition may become strongly female biased. This is because the

longevity of females is slightly greater than that of males. Such a bias is only likely to occur to any appreciable extent in populations that have a single generation each year.

Third, the sex ratio of some species of ladybird may be female biased due to the occurrence of male-killing bacteria. A number of species of coccinellid are known to be infected by endosymbiotic bacteria that kill male embryos but not female embryos. This may result in a female bias in the population sex ratio. For example, about 7% of *A. bipunctata* in Cambridge are infected with a male-killing *Rickettsia*-like bacterium (Hurst *et al.*, 1992; Werren *et al.*, 1994). This results in a pupal population comprising 54% females and 46% males (Hurst *et al.*, 1993). Similarly, some 30% of females in some Russian populations of *A. bipunctata* are infected with a spiroplasma male-killer (Zakharov *et al.*, in prep.; Hurst *et al.*, in prep.). In these populations the proportion of females is about 60%. The bias may be even higher in Japanese populations of *H. axyridis*, where up to 48% of females may be infected by a male-killer (probably a spiroplasma) (Majerus *et al.*, in prep.). The female biases in these populations obviously provide conditions in which females may not encounter males as often as they would were the population sex ration 1:1.

### Conclusion

In most ladybirds, conspecific mate recognition appears to be primarily a function of females. Females that are in reproductive condition and have not been denied access to males for a significant period, vigorously reject non-conspecific males. However, if females are denied access to males for a substantial period, their species recognition system appears to become overridden by their drive to mate. In such circumstances, females may accept a male of another species both in the laboratory and in the field. The male role in species recognition appears to be less important. Observations suggest that male ladybirds in reproductive condition attempt to mount any others they come into contact with irrespective of their sex, species or state of health. Inappropriate encounters (i.e. those with other males, or non-conspecific females) are, in general, only broken off in response to rejection behaviour, or in some cases possibly a lack of an appropriate stimulus from the female.

It is probable that the conditions required to induce females to accept non-conspecific males are rare in the wild. However, they may occur either when a species occurs at very low density, or when the overwintering generation begins to die off prior to the eclosion of the new generation of adults. It is also possible that in species bearing male-killing endosymbionts at high frequency, the sex ratio becomes sufficiently female biased to reduce the encounter rate between females and conspecific males to a level where hybrid matings become a more common occurrence.

One important consequence of the occurrence of inter-specific hybrid matings is that they will allow the transmission of sexually transmitted

diseases between species. For example, inter-specific hybridisation may be implicated in the finding that the sexually transmitted mite *Coccipolipus hippodamiae* infests a wide range of coccinellid species (Webberley *et al.*, in prep.).

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### References

- Donisthorpe, H.St.J.K., 1920. The myrmecophilous ladybird *Coccinella distincta*, Fald., its life-history and association with ants. *Entomologist's Record and Journal of Variation*, **32**: 1-3.
- Hodek, I., 1973. *Biology of Coccinellidae*. Junk: The Hague; Academia: Prague.
- Hurst, G.D.D., Majerus, M.E.N. & Walker, L.E., 1992. Cytoplasmic male killing elements in *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae). *Heredity*, **69**: 84-91.
- Hurst, G.D.D., Majerus, M.E.N. & Walker, L.E., 1993. The importance of cytoplasmic male killing elements in natural populations of the two-spot ladybird, *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae). *Biological Journal of the Linnean Society* **49**: 195-202.
- Hurst, G.D.D., Purvis, E.L., Sloggett, J.J. & Majerus, M.E.N., 1994. The effect of infection with male-killing *Rickettsia* on the demography of female *Adalia bipunctata* L. (two-spot ladybird). *Heredity*, **73**: 309-316.
- Hurst, G.D.D., Sharpe, R.G., Broomfield, A.H., Walker, L.E., Majerus, T.M.O., Zakharov, I.A. & Majerus, M.E.N., 1995. Sexually transmitted disease in a promiscuous insect, *Adalia bipunctata* L. *Ecological Entomology*, **20**: 230-236.
- Hurst, G.D.D., Von de Schulenberg, H.G., Walters, R., Walker, L.E., Majerus, T.M.O., Ashburner, M. & Majerus, M.E.N., in prep. Group VI spiroplasma associated with male-killing in Russian populations of *Adalia bipunctata* L.
- Husband, R.W., 1984. The taxonomic position of *Coccipolipus* (Acarina: Podapolipidae), a genus of mites which are parasites of ladybird beetles. In *Acarology VI, Volume I*, (Eds. Griffiths, D.A. & Bowman, C.E.) pp. 328-336. Ellis Harwood: Chichester.
- Ireland, H., Kearns, P.W.E. & Majerus, M.E.N., 1986. Interspecific hybridisation in the Coccinellidae: some observations on an old controversy. *Entomologist's Record and Journal of Variation*, **98**: 181-185.



- Majerus, M.E.N., 1989. *Coccinella magnifica* Redtenbacher – a myrmecophilous ladybird. *British Journal of Entomology & Natural History*, **1**: 1-19.
- , 1994. *Ladybirds (No. 81 New Naturalist Series)*. Harper Collins, London.
- Majerus, M.E.N. & Kearns, P.W.E., 1989. *Ladybirds (No. 10 Naturalist' Handbook Series)*. Richmond Publishing, Slough.
- Majerus, M.E.N., Kearns, P.W.E., Forge, H. & Ireland, H., 1989. Ladybirds as teaching aids: I: Collecting and culturing. *Journal of Biological Education*, **23**: 85-95.
- Majerus, T.M.O., Majerus, M.E.N., Knowles, B., Wheeler, J., Betrand, D., Kuznetsov, V., Ueno, H. & Hurst, G.D.D., in prep. Variation in male killing behaviour in two populations of the ladybird *Harmonia axyridis* (Col.: Coccinellidae).
- Mayr, E., 1963. *Animal Species and Evolution*. Harvard University Press, Cambridge, Massachusetts.
- McDaniel, B. & Morrill, W., 1969. A new species of *Tetrapolipus* from *Hippodamia convergens* from South Dakota (Acarina: Podapolipidae). *Annals of the Entomological Society of America*, **62**: 1456-1458.
- Newman, L.H., 1965. *Hawkmoths of Great Britain and Ireland*. Cassell, London.
- O'Donald, P. & Majerus, M.E.N., 1992. Non-random mating in the two-spot ladybird, *Adalia bipunctata*. III. New evidence of genetic preference. *Heredity*, **61**: 521-526.
- Pontin, A.J., 1960. Some records of predators and parasites adapted to attack aphids attended by ants. *Entomologists Monthly Magazine*, **95**: 154.
- Ransford, M.O. & Majerus, M.E.N., in prep. Male copulatory behaviour and strategies in *Adalia bipunctata* (Coleoptera: Coccinellidae).
- Webberley, K.M., Hurst, G.D.D., Purvis, E.L. & Majerus, M.E.N., in prep. Infestation of coccinellids by the mite *Coccipolipus hippodamiae* (Acarina: Podapolipidae).
- Werren, J.H., Hurst, G.D.D., Zhang, W., Breeuwer, J.A.J., Stouthamer, R. & Majerus, M.E.N., 1994. Rickettsial related male-killing in the ladybird beetle (*Adalia bipunctata*). *Journal of Bacteriology*, **176**: 388-394.
- Zakharov, I.A., Hurst, G.D.D., Chernyshova, N.E. & Majerus, M.E.N., in prep. The maternally inherited male-killing bacterium in the Petersburg population of *Adalia bipunctata* does not belong to the genus *Rickettsia*. *Russian Journal of Genetics*.

### Some notable Devon Lepidoptera records

Specimens of *Pediasia contaminella* Hb. (Pyralidae) were found on the Maer Local Nature Reserve, Exmouth, East Devon on 20.vii.1996, during a moth event that was being run by East Devon District Council with myself operating the moth traps. This species has been recorded from Dawlish Warren in the past but this locality is in a different tetrad in the SY section of the OS map, whereas the old record is in the SX section.

A specimen of *Ancylosis oblitella* Zell. (Pyralidae) was taken on Dawlish Warren, South Devon on 19.vii.1996 during a routine moth recording session. A second example was later captured on the Axmouth Saltings on 4.viii.1996. I understand that there have been very few records of this species in the past.

A specimen of the Lace Border *Scopula ornata* Scop. (Geometridae) was captured in the Rothamsted trap in Yarner Wood on 20.viii.1984. The